

What is claimed is:

1           1.    A method for detecting nucleic acid target sequences in a sample  
2 comprising:  
3           contacting a sample of nucleic acids with an oligonucleotide probe  
4           under conditions favorable for hybridization, the oligonucleotide probe having  
5           a sequence at least partially complementary to a target nucleic acid sequence  
6           to be detected, the oligonucleotide probe including a fluorescent reporter  
7           molecule and a quencher molecule capable of quenching the fluorescence of  
8           said reporter molecule, said oligonucleotide probe existing in at least one  
9           single-stranded conformation when unhybridized where said quencher  
10          molecule quenches the fluorescence of said reporter molecule, said  
11          oligonucleotide probe existing in at least one conformation when hybridized to  
12          said target polynucleotide where the fluorescence intensity of said reporter  
13          molecule when said oligonucleotide probe is hybridized to said target  
14          polynucleotide is greater than the fluorescence intensity of said reporter  
15          molecule when said oligonucleotide probe is not hybridized to said target  
16          polynucleotide;  
17 and  
18          monitoring the fluorescence of said reporter molecule, an increase in the  
19          fluorescence intensity of the reporter molecule indicating the presence of the  
20          target sequence.

1           2.    The method according to claim 1 wherein the fluorescence  
2           intensity of said reporter molecule when said oligonucleotide probe is hybridized  
3           to said target polynucleotide is at least about a factor of 6 greater than the  
4           fluorescence intensity of said said reporter molecule when said oligonucleotide  
5           probe is not hybridized to said target polynucleotide.

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target nucleic acid sequence to be detected, the oligonucleotide probe including a fluorescent reporter molecule and a fluorescent quencher molecule capable of quenching the fluorescence of said reporter molecule, said oligonucleotide probe existing in at least one single-stranded conformation when unhybridized where said quencher molecule quenches the fluorescence of said reporter molecule, said oligonucleotide probe existing in at least one conformation when hybridized to said target polynucleotide where the fluorescence of said reporter molecule is unquenched, the fluorescence intensity of said reporter molecule being greater than the fluorescence intensity of said quencher molecule when said probe is hybridized to said target polynucleotide;

and

monitoring the fluorescence of said reporter molecule, an increase in the fluorescence intensity of the reporter molecule indicating the presence of the target sequence.

38. The method according to claim 37 wherein the fluorescence intensity of said reporter molecule is at least about a factor of 3.5 greater than the fluorescence intensity of said quencher molecule when said probe is hybridized to said target polynucleotide.

39. A method for detecting nucleic acid target sequences in a sample comprising:

contacting a sample of nucleic acids with an oligonucleotide probe attached to a solid support under conditions favorable for hybridization, the oligonucleotide probe having a sequence at least partially complementary to a target nucleic acid sequence to be detected, the oligonucleotide probe including a fluorescent reporter molecule and a fluorescent quencher molecule capable of quenching the fluorescence of said reporter molecule.

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1 said oligonucleotide probe existing in at least one single-stranded  
2 conformation when unhybridized where said quencher molecule quenches  
3 the fluorescence of said reporter molecule, said oligonucleotide probe existing  
4 in at least one conformation when hybridized to said target polynucleotide  
5 where the fluorescence of said reporter molecule is unquenched, the ratio of  
6 the fluorescence intensities of said reporter molecule to said quencher  
7 molecule when said probe is hybridized to said target polynucleotide is  
8 greater than the ratio of the fluorescence intensities of said reporter molecule  
9 to said quencher molecule when said probe is single-stranded;  
10 and  
11 monitoring the fluorescence of said reporter molecule, an increase in the  
12 fluorescence intensity of the reporter molecule indicating the presence of the  
13 target sequence.

2  
40. The method according to claim ~~39~~<sup>1</sup> wherein the the ratio of the  
1 fluorescence intensities of said reporter molecule to said quencher molecule  
2 when said probe is hybridized to said target polynucleotide is at least about a  
3 factor of 6 greater than the ratio of the fluorescence intensities of said reporter  
4 molecule to said quencher molecule when said probe is single-stranded.  
5

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3. The method according to claim 1 wherein said reporter molecule is separated from said quencher molecule by at least about 15 nucleotides.

4. The method according to claim 3 wherein said reporter molecule is separated from said quencher molecule by between about 15 and 60 nucleotides.

5. The method according to claim 1 wherein said reporter molecule is separated from said quencher molecule by at least about 18 nucleotides.

6. The method according to claim 5 wherein said reporter molecule is separated from said quencher molecule by between about 18 and 30 nucleotides.

7. The method according to claim 1 wherein the reporter molecule is attached to a 3' terminal nucleotide of the probe.

8. The method according to claim 7 wherein the quencher molecule is attached to a 5' terminal nucleotide of the probe.

9. The method according to claim 1 wherein the reporter molecule is attached to a 5' terminal nucleotide of the probe.

10. The method according to claim 9 wherein the quencher molecule is attached to a 3' terminal nucleotide of the probe.

11. The method according to claim 1 wherein the quencher molecule is attached to a 3' terminal nucleotide of the probe.

1 12. The method according to claim 1 wherein the quencher molecule is  
2 attached to a 5' terminal nucleotide of the probe.

1 13. The method according to claim 1 wherein said nucleic acid  
2 polymerase is a thermostable nucleic acid polymerase.

1 14. The method according to claim 1 wherein said reporter molecule is  
2 a fluorescein dye and said quencher molecule is a rhodamine dye.

1 15. A method for detecting nucleic acid target sequences in a sample  
2 comprising:

3 contacting a sample of nucleic acids with an oligonucleotide probe  
4 under conditions favorable for hybridization, the oligonucleotide probe having  
5 a sequence at least partially complementary to a target nucleic acid sequence  
6 to be detected, the oligonucleotide probe including a fluorescent reporter  
7 molecule and a fluorescent quencher molecule capable of quenching the  
8 fluorescence of said reporter molecule, said oligonucleotide probe existing in  
9 at least one single-stranded conformation when unhybridized where said  
10 quencher molecule quenches the fluorescence of said reporter molecule, said  
11 oligonucleotide probe existing in at least one conformation when hybridized to  
12 said target polynucleotide where the fluorescence of said reporter molecule is  
13 unquenched, the fluorescence intensity of said reporter molecule being  
14 greater than the fluorescence intensity of said quencher molecule when said  
15 probe is hybridized to said target polynucleotide;

16 and

17 monitoring the fluorescence of said reporter molecule, an increase in the  
18 fluorescence intensity of the reporter molecule indicating the presence of the  
19 target sequence.

1 16. The method according to claim 15 wherein the fluorescence  
2 intensity of said reporter molecule is at least about a factor of 3.5 greater than  
3 the fluorescence intensity of said quencher molecule when said probe is  
4 hybridized to said target polynucleotide.

1 17. A method for detecting nucleic acid target sequences in a sample  
2 comprising:

3 contacting a sample of nucleic acids with an oligonucleotide probe  
4 under conditions favorable for hybridization, the oligonucleotide probe having  
5 a sequence at least partially complementary to a target nucleic acid sequence  
6 to be detected, the oligonucleotide probe including a fluorescent reporter  
7 molecule and a quencher molecule capable of quenching the fluorescence of  
8 said reporter molecule, said oligonucleotide probe existing in at least one  
9 single-stranded conformation when unhybridized where said quencher  
10 molecule quenches the fluorescence of said reporter molecule, said  
11 oligonucleotide probe existing in at least one conformation when hybridized to  
12 said target polynucleotide where the fluorescence of said reporter molecule is  
13 unquenched, the ratio of the fluorescence intensities of said reporter molecule  
14 to said quencher molecule when said probe is hybridized to said target  
15 polynucleotide is greater than the ratio of the fluorescence intensities of said  
16 reporter molecule to said quencher molecule when said probe is single-  
17 stranded;

18 and

19 monitoring the fluorescence of said reporter molecule, an increase in the  
20 fluorescence intensity of the reporter molecule indicating the presence of the  
21 target sequence.

1 18. The method according to claim 17 wherein the ratio of the  
2 fluorescence intensities of said reporter molecule to said quencher molecule

1 when said probe is hybridized to said target polynucleotide is at least about a  
2 factor of 6 greater than the ratio of the fluorescence intensities of said reporter  
3 molecule to said quencher molecule when said probe is single-stranded.

1 19. A method for detecting nucleic acid target sequences in a sample  
2 comprising:

3 contacting a sample of nucleic acids with an oligonucleotide probe  
4 attached to a solid support under conditions favorable for hybridization, the  
5 oligonucleotide probe having a sequence at least partially complementary to a  
6 target nucleic acid sequence to be detected, the oligonucleotide probe  
7 including a fluorescent reporter molecule and a quencher molecule capable of  
8 quenching the fluorescence of said reporter molecule, said oligonucleotide  
9 probe existing in at least one single-stranded conformation when  
10 unhybridized where said quencher molecule quenches the fluorescence of  
11 said reporter molecule, said oligonucleotide probe existing in at least one  
12 conformation when hybridized to said target polynucleotide where the  
13 fluorescence intensity of said reporter molecule when said oligonucleotide  
14 probe is hybridized to said target polynucleotide is greater than the  
15 fluorescence intensity of said reporter molecule when said oligonucleotide  
16 probe is not hybridized to said target polynucleotide;

17 and

18 monitoring the fluorescence of said reporter molecule, an increase in the  
19 fluorescence intensity of the reporter molecule indicating the presence of the  
20 target sequence.

1 20. The method according to claim 19 wherein the fluorescence  
2 intensity of said reporter molecule when said oligonucleotide probe is hybridized  
3 to said target polynucleotide is at least about a factor of 6 greater than the

1 fluorescence intensity of said reporter molecule when said oligonucleotide probe  
2 is not hybridized to said target polynucleotide.

1 21. The method according to claim 19 wherein said reporter molecule  
2 is separated from said quencher molecule by at least about 15 nucleotides.

1 22. The method according to claim 21 wherein said reporter molecule  
2 is separated from said quencher molecule by between about 15 and 60  
3 nucleotides.

1 23. The method according to claim 19 wherein said reporter molecule  
2 is separated from said quencher molecule by at least about 18 nucleotides.

1 24. The method according to claim 23 wherein said reporter molecule  
2 is separated from said quencher molecule by between about 18 and 30  
3 nucleotides.

1 25. The method according to claim 19 wherein the reporter molecule is  
2 attached to a 3' terminal nucleotide of the probe.

1 26. The method according to claim 25 wherein the quencher molecule  
2 is attached to a 5' terminal nucleotide of the probe.

1 27. The method according to claim 19 wherein the reporter molecule is  
2 attached to a 5' terminal nucleotide of the probe.

1 28. The method according to claim 27 wherein the quencher molecule  
2 is attached to a 3' terminal nucleotide of the probe.



